

Ribavirin Small-Particle Aerosol Treatment of Infections Caused by Influenza Virus Strains A/Victoria/7/83 (H1N1) and B/Texas/1/84

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In a double-blind study of influenza in a population of college students in 1984, ribavirin small-particle aerosol treatment of 38 patients (18 treated, 20 control) infected with a new antigenic variant, influenza virus strain A/Victoria/7/83 (H1N1), was associated with statistically significant reductions in the height and duration of fever, systemic symptoms, and virus shedding. Patients received a total of 2.4 g of ribavirin over 42 h during 68 h of hospitalization without any side effects. In addition, in a study of patients infected with influenza virus strain B/Texas/1/84 (seven treated, eight control) treated with ribavirin aerosol showed a trend of more rapid recovery than control patients.

Ribavirin small-particle aerosol treatment of college students with influenza infections has been shown to be effective against the two major influenza virus groups, A and B (6, 7). In 1984, a mixed infection of influenza virus strain B/Texas/1/84 and a new antigenic variant of influenza A, strain A/Victoria/7/83 (H1N1), occurred in this population. In this study, we report the results of a double-blind, randomized evaluation of ribavirin small-particle aerosol treatment of infection with these new variants. The findings indicate a favorable response to treatment of influenza virus strain A/Victoria/7/83 (H1N1) infection, but equivocal results were obtained with the small number of patients with influenza virus strain B/Texas/1/84 infection who were treated similarly.

MATERIALS AND METHODS

Patients. The patient population consisted of students admitted to the Beutell Health Care Center, Texas A & M University, College Station, Tex. As in previous studies, students were invited to participate in the study if they had both an oral temperature of at least 38.3°C and systemic symptoms of influenza that were of less than 24 h duration (4a, 6, 7, 9). Diagnosis of influenza was based on the isolation of virus from a nasal wash specimen.

In the 1984 study, a mixed outbreak of influenza occurred. Of the 59 students enrolled in the study, 38 patients were infected with influenza virus strain A/Victoria/7/83 (H1N1), 15 patients were infected with influenza virus strain B/Texas/1/84, 1 patient was infected with influenza virus strain A (H3N2) and 5 patients did not yield virus. Of the patients infected with influenza virus strain A (H1N1), 18 were treated and 20 were used as controls, whereas 7 of those infected with influenza B virus were treated and 8 were used as controls.

Ribavirin small-particle aerosol treatment. The aerosol-producing system was the same system described previously (7), except that Aridyne 2000 Medical Air Compressors manufactured by Timeter Instrument Corp. were used as the source of compressed air. The aerosol contained an average of 190 µg of ribavirin per liter of air at a flow rate of 12.5 liter/min. The particles had a mass median diameter of 1.6

µm; 95% of the particles had an aerodynamic mass median diameter less than 5 µm. Control subjects were treated with an aerosol of sterile water.

Treatment was begun soon after the patient was admitted and was continued for 16 to 18 h or until 8 a.m. the following morning, whichever came first. The following morning all participants were evaluated, and treatment was then resumed in three, 4-h blocks for a total of 12 h per day. Treatment was continued until 12 noon on day 3. The ribavirin dosage was calculated as follows: the mean daily minute volume (in cubic centimeters per minute per kilogram) was calculated by multiplying the mean daily respiratory rate per minute by the mean tidal volume (5.43 ± 0.44 cm³/kg). Minute volumes (in liters) for each patient each day were estimated by taking 0.19 mg (mean ribavirin aerosol concentration per liter) times 60 min times the number of hours of treatment each day times 0.7 (estimated fraction of inhaled aerosol deposited in the respiratory tract).

Clinical assessment of illness. A detailed clinical assessment of each patient was made on admission and daily at 8:00 a.m. and 4:00 p.m. thereafter until discharge. Patients were examined in a separate examining room by physicians who did not know their treatment status. Patients were not informed of their treatment status until they were discharged from the hospital. The nursing staff was not informed as to the treatment status. At the conclusion of each examination, the patient's illness was scored on a range of 0 to 3+ (most severe) according to the categories of rhinitis, pharyngitis, tracheobronchitis, pneumonia, and systemic illness. As in previous studies, systemic illness, characterized by prostration, anorexia, headache, and muscle aches, was the most prominent feature of the illness. Temperatures were measured orally every 4 h by the nursing staff. Patients were considered afebrile when their temperatures returned to sustained levels of less than 37.8°C. Antipyretics were not routinely available; however, patients could receive acetaminophen (500 mg) for temperatures exceeding 39.7°C. A greater number of patients with influenza A required acetaminophen in this study than in previous studies, reflecting the increased severity of influenza illness associated with the new antigenic variant. A total of 12 of 20 control and 6 of 18 treated patients received acetaminophen. Neither the pro-

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portion of patients nor the total dosage of drug received was statistically significant between the two groups.

The following clinical chemical values were determined by automated methodology on blood specimens obtained at admission, at discharge, and 8 days and 1 month after discharge: sodium, potassium, chloride, calcium, phosphorus, urea, creatinine, uric acid, cholesterol, triglycerides, total protein, globulin, albumin, glucose, total bilirubin, direct and indirect bilirubin, alkaline phosphatase, aspartate aminotransaminase, alanine aminotransaminase, lactic dehydrogenase, and γ -glutamyl transaminase.

Hematologic studies consisted of the determination of percent hematocrit, grams of hemoglobin, reticulocyte count, leukocyte count with a differential cell count, and platelet count. In addition, female patients in the study denied having symptoms of early pregnancy and had negative urinary screening tests for pregnancy.

Virus isolation, identification, and quantification. A nasal wash specimen was collected on admission from every patient who entered the study for virus identification and was transported in veal infusion broth. In addition, for virus quantification nasal wash specimens (lactated Ringers solution supplemented with veal infusion broth for storage at -70°C) were taken on admission and twice daily thereafter for 3 days. A follow-up nasal wash was obtained 7 to 10 days later. A portion of the initial veal infusion broth sample was inoculated into Madin-Darby canine kidney, rhesus monkey continuous (designated as LLC), human epithelial carcinoma (HEp-2), and human embryo fibroblast (WI-38) cell cultures, and standard diagnostic procedures, including immunofluorescence, were used to identify individual subtypes (1, 3). In addition, postinfection ferret antisera to selected influenza B variants and a battery of mouse monoclonal antibodies to the hemagglutinin of influenza B/Oregon/80 were used to finally identify the virus strain. Virus quantification was performed in 24-well plates containing monolayers of Madin-Darby canine kidney cells as previously described (6). Virus titers, in 50% tissue culture infective doses per milliliter of original nasal wash, were calculated by the Karber method (8).

Hemagglutination-inhibition-antibody titration. Serum specimens from acutely ill and convalescent patients were titrated by standard methods as previously described for hemagglutination-inhibition titers (2, 4). Five antigens, A/England/333/80 (H1N1), A/Dunedin/6/83 (H1N1), A/Victoria/7/83 (H1N1), B/Texas/1/84, and B/Singapore/222/79, were used in the tests.

Analysis of patient data. Data analysis was performed with the aid of the clinical information data management and analysis system. *P*-values are based on analysis of these data by the Wilcoxon rank sum or the Student *t*-test (two-tailed) as indicated.

RESULTS

Infections with influenza virus strain A/Victoria/7/83 (H1N1). Of the 54 patients infected with influenza virus, 38 were infected with the A/Victoria/7/83 strain. A total of 18 patients were treated with ribavirin aerosol, and 20 patients were given sterile water aerosol as controls. There were 8 females (4 treated, 4 control) and 30 males (14 treated, 16 control); 2 treated and 7 control patients reportedly had been vaccinated against influenza more than 24 months before admission to this study. A total of 26 students reported having had influenza, but there was no statistically significant difference in the number or time of prior infection between patients in either the treated or control group (*P* =

0.626). None of the differences in demographic parameters was statistically significant.

Febrile response. Ribavirin small-particle aerosol treatment reduced both the duration of fever ($\geq 37.8^{\circ}\text{C}$) (Table 1) and the mean maximum 12-h temperature. From the start of treatment, fever persisted 18.7 h longer in control than in treated patients (*P* = 0.004). At 24, 36, and 48 h after the start of the treatment, patients receiving ribavirin aerosol had statistically significant reductions in the mean maximum temperature compared with control patients (*P* = 0.024, 0.015, and 0.004, respectively).

Quantification of virus shedding in nasal wash specimens. Virus titers were determined on nasal wash specimens taken at the time of admission and twice daily thereafter. Within 12 h from the start of treatment, mean virus titers in treated patients were lower than on admission, whereas patients receiving sterile water did not show any reduction in virus titers until at least 24 to 36 h after admission (Fig. 1). The reduction in virus concentration was statistically significant between 12 and 48 h posttreatment (*P* = 0.001 to 0.048).

Systemic illness. Systemic illness, characterized by prostration, anorexia, headache, and muscle aches, was the most pronounced symptom and was reduced in treated as compared with control patients by 24 h posttreatment (*P* = 0.054) (Table 2). This difference in systemic illness scores persisted through 36 h. Rhinitis, pharyngitis, and tracheo-bronchitis were never severe in either group of patients.

Influenza antibody titers. Of the 38 patients with influenza identified by virus isolation, 37 demonstrated a fourfold or greater rise in antibody titer in serum to the new antigenic type A/Victoria/7/83, whereas only 30 demonstrated a rise to the previously circulating antigen A/England/333/80 (*P* = 0.012; chi-square analysis). The geometric mean antibody titers for either acute or convalescent sera were not different for either antigen in treated or control patients (Table 3). However, there was a statistically significant greater fold rise in antibody titer with the A/Victoria/7/83 antigen (*P* = 0.005; the Wilcoxon rank sum test, two-tailed).

Ribavirin aerosol dosage. Individual daily treatment periods were similar in treated and control patients with a total of about 42 h (Table 4). The 18 patients treated with ribavirin received a total of 2.4 g of drug during their 41.9 h of treatment over the 3 to 4 days of treatment. This dosage of ribavirin can be calculated to have a mean concentration of 34.9 mg/kg or 0.83 mg/kg per h of treatment. There was no toxicity observed.

Hematological findings. A slight but significant increase in hematocrit was seen in both treated and control patients at the time of discharge from the hospital as compared with

TABLE 1. Timing of events in patients with influenza virus strain A/Victoria/7/83 (H1N1) infections

Event	Time (h \pm SEM) for the following patients:		<i>P</i> value ^b
	Treated (18) ^a	Control (20) ^a	
Onset of systemic illness to:			
Admission	17.8 \pm 1.3	15.4 \pm 1.3	0.209
Start of Treatment	19.0 \pm 1.3	16.5 \pm 1.3	0.171
Afebrile ($<37.8^{\circ}\text{C}$)	49.0 \pm 4.8	65.2 \pm 3.8	0.011
Discharge	85.6 \pm 2.1	83.8 \pm 1.8	0.3
Start of treatment to afebrile state	29.9 \pm 5.0	48.6 \pm 3.8	0.004

^a Number of patients is indicated in parentheses.

^b The Student *t* test, two tailed.

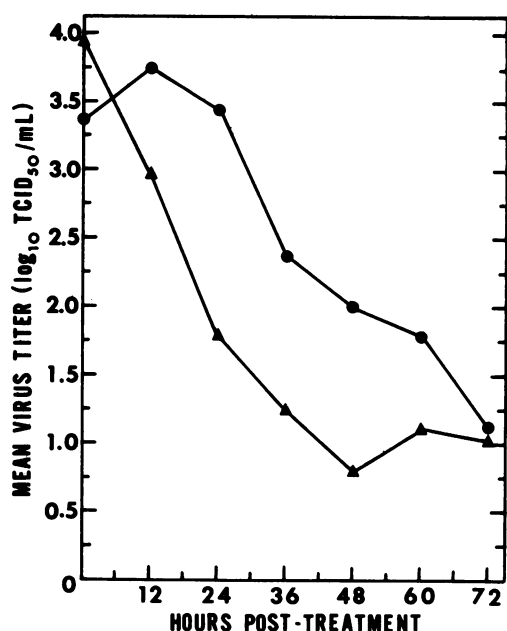


FIG. 1. Mean virus titers at 12-h intervals from the start of treatment in patients infected with influenza virus strain A/Victoria/7/83 (H1N1). At 0, 12, 24, 36, 48, 60, and 72 h, *P* values (the Student *t* test, two-tailed) were 0.120, 0.048, 0.001, 0.009, 0.007, 0.129, ≥ 0.3 , respectively. Symbols: ●, control patients; ▲, treated patients. TCID₅₀, 50% tissue culture infective dose.

mean values at admission and at 1 week and 1 month after admission (Fig. 2). The total leukocyte count in treated and control patients was about 7,000/mm³ at admission and dropped significantly ($P < 0.02$) at the time of discharge about 3 days later. The reduction in counts was greatest among control patients. Thereafter, the counts returned to normal values. Changes in total leukocyte counts were principally contributed by a major decline in the number of polymorphonuclear cells, which was greatest among control patients ($P = 0.06$). In treated patients, these counts returned to normal in 1 week. Values in control patients remained low at 1 week (treated versus control; $P = 0.03$). At 1 month, treated and control values were similar. Not shown was a significant reduction in reticulocyte counts, which was from about 1.2% in treated and control patients at admission to about 0.8% at the time of discharge from the hospital. These counts returned to normal ranges by 1 month after admission. Platelet counts did not show significant fluctuations in treated or control patients.

Influenza virus strain B/Texas/1/84 infection. Of the 54 patients with influenza infection, 15 had influenza B virus

TABLE 2. Mean systemic symptom score based on 12 h intervals from start of treatment in patients with influenza virus strain A/Victoria/7/83 (H1N1) infection

Patient (no.) and <i>P</i> value	Range of severity ^a at the following times (h) post treatment:						
	0	12	24	36	48	60	72
Treated (18)	2.8	2.6	1.8	1.4	1.1	0.9	0.6
Control (20)	2.8	2.6	2.3	2.1	1.6	1.2	0.4
<i>P</i> value ^b	0.878	0.839	0.054	0.052	0.095	0.269	0.639

^a Range of severity: 0 to 3+ (severe).

^b The Wilcoxon rank sum test, two-tailed.

TABLE 3. Geometric mean serum hemagglutination-inhibition antibody titers in serum to influenza virus strains A/England/333/80 (H1N1) and A/Victoria/7/83 (H1N1)

Patient (no.) and <i>P</i> value	Geometric mean titer of antibody to the following strains in the indicated stages of illness:			
	A/England/333/80 (H1N1)		A/Victoria/7/83 (H1N1)	
	Acute	Convalescent	Acute	Convalescent
Treated (18)	4.2	37.3 (8.9) ^a	4.2	94.4 (22.3)
Control (20)	4.5	32.0 (7.1)	4.3	78.8 (18.3)
<i>P</i> value ^b	0.882	0.788	0.973	0.630

^a Fold rise in geometric mean antibody titer is indicated in parentheses.

^b The Wilcoxon rank sum test, two-tailed.

isolated from their initial nasal wash specimen. In this small group (seven control, eight treated), the time from the onset of systemic illness to the start of treatment was more than 5 h longer in control than in treated patients (20.5 ± 2.2 , 15.4 ± 2.1 h, respectively). The time of onset of systemic illness or from the start of treatment to a sustained temperature of less than 37.8°C (i.e., afebrile) was less in treated patients (16.1 versus 11.0 h, respectively), but the differences were not significant. In addition, the number of patients with a duration of fever greater than 50 h was seven of seven patients for the control group and only four of eight patients for the treated group ($P = 0.077$; the Fisher exact test). These results, along with the reduced mean maximum temperature of treated patients at 24 h posttreatment ($P = 0.065$), suggest a therapeutic effect for ribavirin aerosol in this group.

Hematological changes in influenza B patients closely resembled those described for patients infected with influenza virus strain A/Victoria/7/83 (H1N1). Specifically, polymorphonuclear cell counts diminished from admission to discharge, whereas lymphocyte counts increased in this interval and returned to normal ranges by 1 month after admission. The reduction of reticulocyte counts at discharge was similar in degree to that in patients infected with influenza virus strain A/Victoria/7/83 (H1N1) (data not shown).

Clinical chemical findings in patients infected with influenza viruses A and B. Among 23 clinical chemical tests performed during and after illness, there were no significant differences between treated and control patients. Specifically, bilirubin and liver enzymes tests did not vary from normal in treated and control patients.

DISCUSSION

The present study has shown a consistent and substantial therapeutic effect of ribavirin aerosol in the treatment of infection caused by a new variant of influenza virus, strain A/Victoria/7/83 (H1N1). Previous studies have shown that ribavirin is active in the treatment of infections caused by

TABLE 4. Mean ribavirin dosage in treated patients infected with influenza A virus

Day	Ribavirin dosage		Mean time treated (h)	No. of patients
	mg/day	mg/kg/day		
0	543	7.8	8.3	18
1	1011	14.8	17.9	18
2	647	9.5	11.9	18
3	189	2.8	3.8	2

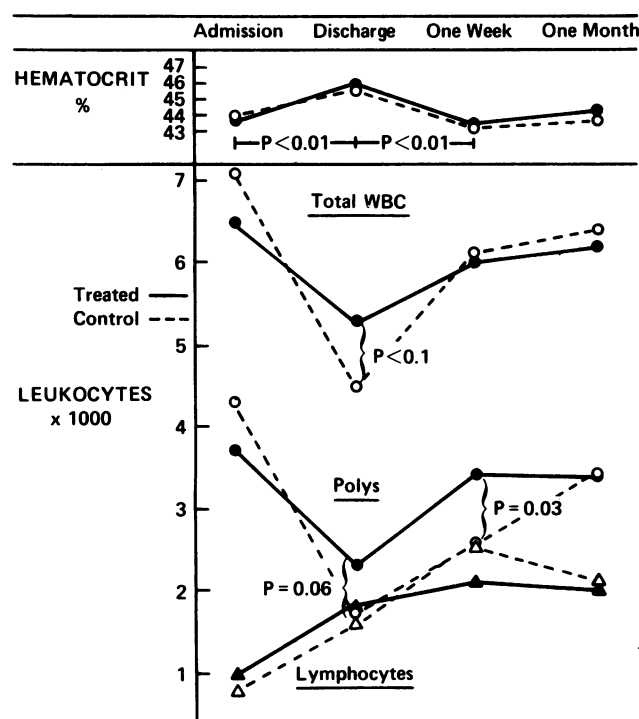


FIG. 2. Mean hematocrit and leukocyte (WBC) absolute counts in treated and control patients with influenza virus strain A/Victoria/7/83 (H1N1) infection. In addition to probabilities of differences between treated and control patients indicated on the figure, the wide variations at different time periods within treated and control groups were highly significant ($P < 0.01$). For example, the drop in absolute polymorphonuclear (Polys) cell counts from admission to discharge in treated patients was highly significant ($P = 0.001$), and the increase in polymorphonuclear cell counts in treated patients from discharge to 1 week was highly significant ($P = 0.01$). Open symbols represent control patients; closed symbols represent treated patients.

influenza virus strains A/England/333/80 (H1N1) in 1981 (6) and 1982 (9), influenza virus strain A/Bangkok/1/79 (H3N2) in 1983 (4a), and influenza virus strain B/Singapore/222/79 in 1982 (7). A trend of therapeutic effect was also seen in 1984 in a study of a small number of cases of infection with influenza virus strain B/Texas/1/84. No effect was observed in a study of disease caused by infection with influenza virus strain A/England/333/80 (H1N1) in 1983 (Smith et al., ed., *Clinical Aspects of Ribavirin*, in press).

The influenza virus strain A (H1N1) disease encountered in the past 4 years has been a mild illness, with fever occurring in control patients receiving sterile water aerosol, persisting for an average of 40 h from the start of treatment. The period from the onset of illness to the start of treatment averaged about 15 h in all groups. Fever in treated patients persisted 28 h from the start of treatment. However, in the 1983 study (4a), in which no effect was detected, duration of fever from the start of treatment was 30.3 and 33.5 h in treated and control patients, respectively. When the studies with positive results only were considered, it was found that fever in control patients (start of treatment to afebrile state) persisted for an average of 42 h, whereas it lasted for only 27.5 h in treated patients. Thus, with the temperatures of patients available in these studies, it can be determined that a difference of about 15 h in the duration of fever between treated and control patients will be required for a therapeutic

effect to be demonstrated. In the present study this result was achieved. In 1981, this result was achieved because treated patients had fever for only 21 h, whereas in control patients, fever lasted for 35.5 h.

This degree of variation in duration of fever between treated and control patients may be consistent with the concept that we have observed a fairly uniform type of illness from year to year. In support of this is the finding that the duration of fever from start of treatment in influenza virus strains A/Bangkok/1/79 (H3N2) and B/Singapore/222/79 infections lasted 55 h in control patients (4a, 7). The use of duration of fever as a measure of severity seems justified because virus shedding patterns and symptomatology also correlated with this index.

The polymorphonuclear cell counts in treated and control patients with influenza virus strain A/Victoria/7/83 (H1N1) infection can also be interpreted to indicate a therapeutic effect of ribavirin aerosol. The counts in treated patients dropped less during treatment than they did in controls, suggesting that treatment minimized the suppressive effect of influenza virus infection on these cells. At 1 week after admission the polymorphonuclear cell counts of treated patients were essentially normal, whereas counts in control patients remained low ($P = 0.03$). Total leukocyte counts also reflected these differences. Changes in the other leukocyte count series were similar in treated and control patients.

The percent hematocrit in treated and control patients was increased significantly at discharge over the value at admission and the values at 1 week and 1 month after admission. This increase may have been due to the nearly continuous use of a face mask in treated and control patients which interfered with the oral intake of fluids. Other explanations should also be sought for this finding because it could also represent dehydration as a consequence of infection, even though it occurred during a time when defervescence and recovery was occurring and oral intake was unrestricted.

A significant reduction in the percentage of circulating reticulocytes was noted in an earlier study (6), and this difference was again observed. The change occurred in both treated and control patients. Changes in blood counts of patients with influenza virus B were similar to those with influenza virus A infection.

As in previous studies, there was no laboratory or clinical evidence of toxicity of ribavirin small-particle aerosol. The present report thus extends the findings of the safety and therapeutic effectiveness of ribavirin aerosol in the treatment of infections caused by influenza viruses A/Victoria/7/83 (H1N1) and B/Texas/1/84.

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